THE TRANSFORMATION AND TRANSLOCATION OF THE FOODS IN THE SEEDLINGS OF ZEA MAYS

by

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INTRODUCTION

The purpose of this problem was to determine by chemical analyses some of the changes in the proteins, carbo-

hydrates and fats that occur in the corn grain during its germination and to investigate the distribution of these organic materials in the seedling at different stages of its growth. Although considerable work has been done on the analysis of the seeds and seedlings of the various cereals, there has been but little investigation of the chemical changes occurring during the germination of the corn grain and our knowledge on this subject is very meager.

On that account it was considered advisable to investigate this point with the hope of adding a little more definite information to our knowledge of this important crop plant.

This work was undertaken at the suggestion of Dr. E. C. Miller of the Department of Botany and Plant Pathology and I am indebted to him for guidance and assistance in the experiment. I wish also to thank Dr. H. H. Haymaker of the same department for his interest and advice in the formulation and direction of my course of graduate study.

LITERATURE REVIEW

Proteins. Probably more work has been done on the determinations and characteristics of proteins than of carbohydrates and fats. Chittenden and Osborne (1) found that the maize kernel contains three globulins, one or more albumins, and an alcohol soluble protein. The globulin

obtained by extraction with ten per cent solution of sodium chloride was a mixture of two or more dissimilar globulins, differing from one another both in composition and in coagulation points. When separated, two of the globulins were a myosin-like body and a vitellin-like body. The myosin-like globulin was characterized by containing about 16.8 per cent nitrogen and 1.2 per cent of sulphur. It had a coagulation point, in ten per cent salt solution, of about 70 degrees C. The vitellin-like globulin contained about 18.1 per cent nitrogen and 0.85 per cent sulphur. The third globulin present was characterized by extreme solubility in very dilute salt solutions, separating from such solutions only by prolonged dialysis. An aqueous extract of corn meal, as well as a sodium chloride extract, contained, in addition to the globulins, apparently two albumin-like bodies, more or less coagulable by heat, but unlike in chemical composition. A certain amount of proteose was detected, but was considered to be mainly a product resulting from the hydrolysis of some one or more of the globulins or albumins. Zein, soluble in warm dilute alcohol, but insoluble in water, was found.

Osborne (12) found the following proteins and their amounts in milligrams per 100 grams of yellow corn meal: proteose, 60; maysin, 250; globulin, 40; edestin, 100; zein,

5,000; protein soluble in 0.2 per cent potash, 3,150. The nitrogen in these proteins totaled 1.3809 grams. Undissolved nitrogen amounted to 0.1645 grams, making a total of 1.5454 grams per 100 grams of corn meal. The mean per cent of nitrogen in the maize proteins was 16.057. In a further study of the corn kernel, Osborne (13) found a very small proportion of protein soluble in water or neutral saline solutions, a relatively large amount soluble in strong alcohol, and a considerable quantity insoluble in all neutral solvents but soluble in very dilute alkaline and acid solutions. He found 8.6 per cent of the kernel to be protein.

Hopkins (4) found that the protein in the corn kernel amounted to approximately 11 per cent, the fats to 4.65 per cent, and the carbohydrates to 82.85 per cent.

Miller (9) used sunflower seeds and grew the seeds in thoroughly washed white quartz sand. The vessels containing the seedlings were kept in a greenhouse at a temperature of 65 to 75° F. After the seedlings reached the top of the sand, they were allowed to grow in sunlight, but special precautions were taken to exclude all carbon dioxide so the process of photosynthesis could not proceed, except from the carbon dioxide liberated by the plants in respiration. Miller examined the seeds and five stages of seedlings up to thirteen days.

During the first three days, five-sixths of the sugar content of the cotyledons, one-seventh of the oil, and almost one-fourth of the protein had disappeared. The most marked change in the reserve products occurred between the fifth and tenth days. The protein reserve during the progress of germination was broken up apparently into the ordinary cleavage products which were transported into the roots and hypocotyls, where they were used in the formation of new cells.

Le Clerc and Breazeale (7), working with wheat seedlings, found a gradual decrease of nitrogen in the residual seed, with a gradual increase in the seedling. At the end of 12 days very little was left in the residual seed, but there was about as much in the axes as the original amount in the seed.

Thompson (18) found an increase of amino nitrogen in first-day seedlings of the Alaska pea. He explained this as due, probably, to the decomposition of the proteins present in the original seed. He explained the large increase in the percentage of amino nitrogen in the six-day plant as probably due to the rapid transformations in the seed, and to the requirements to build up material in the leaves of a nitrogenous nature. The total nitrogen remained fairly constant in the natural leaves. As the plant became older there was a decrease in the total and amino nitrogen.

Pettibone and Kennedy (14) grew corn seedlings in tap water. They found that as the total nitrogen of the seed diminished the combined total nitrogen of root and plumule increased, indicating transference of nitrogenous material from the seed to these parts. The sum total of nitrogen in the entire seedling showed a value near that in the unsprouted kernel. Amino acids were found to be present in the flowing sap.

Choate (2) made analyses of Marquis wheat, ungerminated, and germinated, for periods varying from one-half to seven days. She reported the storage proteins of the endosperm to be broken down during germination, and at the end of seven days the nearly exhausted remnant of the endosperm gave only a slight protein reaction. The aleurone layer, however, was still intact and apparently unchanged.

Latshaw and Miller (8) found grain of mature corn plants, harvested when the grain was glazed, to contain 2.15 per cent nitrogen.

Toole (19) found 1.55 to 2.53 per cent of nitrogen in the water extract from embryos of mature corn kernels. He found the proteins of corn to undergo very early dissolution or partial hydrolysis upon germination.

Yocum (20) found nitrogen to be translocated rapidly in the three-day wheat seedling, after which the percentage

remained constant in the axes. The nitrogen content of the seed decreased in proportion to the weight the first nine days after which a more rapid loss occurred.

Jodidi (5) reports 48 per cent of the protein of the corn seed converted into water-soluble, diffusible nitrogen compounds during the first eight days of germination. Jodidi (6) isolated and identified asparagin in etiolated corn seedlings.

Davidson (3) proved to his satisfaction that wheat seedlings either lost or gained nitrogen, depending upon their age and the conditions under which they were grown. He germinated one lot of seeds so that they did not touch each other and another in which they were in direct contact. Both lots were grown floating on distilled water. Seven days from the time the seeds were set to germinate, the nitrogen content of the seedlings were about ten per cent greater than the nitrogen content of the stock seed. He reports indications that the gains in nitrogen were due chiefly to the absorption of nitrogen leached from the ungerminated seeds since the ungerminated seeds lost more nitrogen than did the seedlings.

Newcomb (11) reports no loss of nitrogen during the very earliest stages of growth of sunflower seedlings before the sprouts were more than 1.4 centimeters long. A loss was

obtained in all cases where the sprouts were 4.5 cm. or more in length, the loss of nitrogen ranging from 5 to 29 per cent. With squash seeds and seedlings, a decrease ranging from 6 to 25 per cent was noted. There was a slight increase in the nitrogen content in the seedlings of the peanut.

Stark (17), in experimenting with Midwest and Manchu varieties of soybeans, found no correlation to exist between protein hydrolysis and growth except for very early periods. He also found respiratory intensity unrelated to alpha-amino nitrogen content.

Carbohydrates. Le Clerc and Breazeale (7) found no reducing sugar in the original seed of the wheat, but reported 95 mg. of non-reducing sugar in 100 seeds. During germination the formation of reducing sugar in the seed was rapidly increased up to the sixth day, after which there was a rapid falling off in the amounts of these sugars until at the end of the fifteenth day the residual seed contained but a trace. In the axes, the reducing sugars increased up to about the ninth day, at which time they contained about three times as much of these sugars as the original seed did of hydrolyzable sugar. After this period there was a gradual decrease in reducing sugars in these parts.

Choate (2) found a small amount of sucrose in the endosperm and embryo of Marquis wheat. Reducing sugar appeared in the embryo after 18 hours in the germinator.

Toole (19) detected no reducing sugars in the embryo of the dry grain of wheat, but some sucrose. The presence of reducing sugar was detected about 20 hours after grains were placed in the germinator and in the later stages of germination it ranged in amount from 4.5 per cent to 16.8 per cent. In the embryo sucrose was present in comparatively small and constant amounts during germination. According to the work of Yocum (2) the small amount of sugars normally found in the wheat kernel increased rapidly in the seed during the first six days of germination, during which time nearly three-fourths of the starch had been used. After six days the amount of sugar decreased. While there is a supply of starch in the seed, the sugars are translocated to the plumules and roots more rapidly than they can be used.

Fats. According to Miller (9), the oil in sunflower seedlings is broken down, partly, at least, to free fatty acids and glycerine. The marked increase noted in the amount of sugar during the development of the seedling has its origin in the oily reserve and the sugar thus produced is the material used by the plant for the formation of new cell walls in the growing parts. Miller (10) reports only

5.3 per cent of the original oily reserve remaining in the cotyledons at the end of 15 days. His results showed a gradual but well defined breaking down of the oily material during the early stages of germination.

Le Clerc and Breazeale (7) found in wheat seedlings that 60 to 67 per cent of the original crude fat of the seed remained at the end of 12 days of germination. During this same period the acid, with an original fat content of 30 to 35 per cent, had increased the amount from 70 to 103 per cent.

Poole (19) found that fatty substances in the embryo of the corn kernel amounted to 40 per cent of the dry weight of the embryo. This amount slowly and gradually increased during the growth of the seedling.

Yocum (20) states: "During the early stages of the germination of the wheat seedling, the ether extract disappears from the seed more slowly than the carbohydrates or nitrogen, and accumulates in the plumules and roots more slowly than any other food material investigated."

Shine (15) concludes that all the fat stored in the oily seed is first converted to sugars before being transported. He found no evidence to substantiate the idean of fat movement in plants as fat.

EXPERIMENTAL METHODS

Cultural Methods

Ear corn of the Pride of Saline variety, grown near Manhattan, Kansas, was selected for the experiment. After discarding the tip and butt kernels because of their irregularity in size, a uniform sample of the shelled grain was obtained by hand selection, the small, oversized, or injured seed being removed. In order to eliminate as much as possible the growth of molds, the seeds were treated by immersion for several minutes in a solution composed of one-third ounce of Semesan in a gallon of water. After the seeds were removed from the solution, they were thoroughly rinsed with water to remove the Semesan and thus reduce to a minimum any influence that this chemical might have upon the metabolism of the seedling.

After this treatment the seeds were placed in rag doll germinators and the dolls were saturated with water. These dolls were then placed upon suitable supports in metal ten-gallon cans. Water was placed in the bottom of the cans to a depth of 2 inches, after which they were covered with their lids. During the germination period the dolls were saturated with water every second or third day to insure a

sufficient supply of water for germination and growth. The cans containing the germinators were then placed in the greenhouse at a temperature of 30 to 35° C.

Seedlings were selected for examination at the end of 5, 10 and 14 days. The 5-day seedlings were germinated from February 11 to 16, the 10-day seedlings from March 14 to 24, and the 14-day set from February 24 to March 10, 1931. Upon their removal from the germinators only those seedlings that were of the average size and free from molds were selected for the experiment. The plumules, roots, and mesocotyls were then carefully removed and placed in separate lots. This material was then placed in the drying oven at 100° C. After 24 hours it was removed and ground in a mortar until it would pass through a 40-mesh sieve. The material was then returned to the oven at 100° for a 24-hour period, after which it was bottled and stored in a desiccator until time to be used.

Analytical Methods

The analytical methods used were the methods outlined in the "Official Methods of Analysis of the Association of Official Agricultural Chemists."

Nitrogen. The official Kjeldahl method was used for the nitrogen determination. One-gram samples in triplicate of endosperm, plumule, and root materials were taken while

only one-half gram samples in duplicate of mesocotyl and embryo materials were used. Each sample was placed in a Kjeldahl flask along with a small piece of copper wire, and one teaspoonful of potassium sulphate. Thirty cubic centimeters of concentrated sulphuric acid were then carefully added. Each flask was then heated over a flame, slowly at first, and then with heat increased gradually until material boiled hard. The boiling was continued until one-half hour after the liquid became clear. It was then cooled and 300 cubic centimeters of tap water, a few drops of phenolphthalein, a piece of granulated zinc and fifty cubic centimeters of 45 per cent sodium hydroxide were added.

About 300 cc. from each flask were then distilled over into titration flasks into which had been placed 25 cc. of 1/2n. sulphuric acid, 50 cc. of distilled water, and a few drops of methyl red. The excess acid was then determined by titration with 1/10 sodium hydroxide, and the amount of nitrogen determined.

Sugar Solution. Twenty grams each of plumule, root and endosperm material and 5g gm. of mesocotyl and embryo material were weighed out and transferred to 500 cc. volumetric flasks. About 3 gm. of calcium carbonate were added to each flask in order to neutralize any acid that might be present. Two hundred fifty cc. of 80 per cent alcohol

were then added and the contents shaken thoroughly. The flasks were then put on steam baths and heated for one hour, the loss of the liquid being prevented by a small funnel placed in the neck of each flask. The material was cooled over night and 95 per cent alcohol was added, making it up to the mark, after shaking thoroughly; it was then allowed to settle, after which 350 cc. of the clear solution were pipetted off, transferred to beakers and evaporated down to 100 cc. These samples were then transferred to 250 cc. volumetric flasks and sufficient saturated neutral lead acetate solution was added to precipitate the proteins and tannins. After standing 15 minutes, the contents of the flasks were diluted to the mark with water, mixed thoroughly, and filtered through dry filter paper. Sufficient sodium carbonate was then added to the filtrate to precipitate the excess lead, after which it was again filtered. The final filtrate then was placed in stoppered containers until its sugar content could be determined.

Reducing sugars. Twenty-five cc. portions each of copper sulphate and tartrate solutions were pipetted in a 400 cc. beaker. Twenty-five cc. of the sugar solution and 25 cc. of water were transferred to this beaker, which was then covered with a watch glass. The beaker was heated on gauze over a Bunsen burner, regulated so as to bring the

solution to a boil in four minutes, after which the boiling was continued for two minutes.

The solution was filtered at once through an asbestos mat in a weighed Gooch crucible, using suction. The precipitate was washed with water at 80° C., then with 10 cc. of 95 per cent alcohol, and then 10 cc. of ether. The crucible was then placed in the oven and dried for 30 minutes at 98°C. The crucibles were again weighed and the sugar calculated as d-glucose from the weight of the cuprous oxide precipitate.

Non-reducing Sugars. Fifty cc. of the sugar solution were transferred to a 100 cc. volumetric flask and neutralized with hydrochloric acid. Five cc. of concentrated hydrochloric acid were then added and inversion allowed to proceed on the water bath for fifteen minutes. The solution was then neutralized with sodium carbonate solution, and diluted to the mark. Twenty-five cc. portions were then taken for the determination of the reducing sugars present by means of Fehling's solution.

Starch. Five grams of the dry material were placed in a beaker with 100 cc. cold water and stirred at frequent intervals for one hour. The contents of the beaker were then transferred to a filter and washed with 250 cc. of cold distilled water. The insoluble residue was then heated two and one-half hours with 250 cc. of distilled water and 20 cc.

of dilute hydrochloric acid in a flask provided with a reflux condenser. The solution was then cooled and nearly neutralized with sodium hydroxide and the volume consisted to 250 cc. It was then filtered and 25 cc. portions were used for determination of reducing sugars.

Crude Fats. Two-gram samples were taken in duplicate, extracted with ether for 16 hours, and the fat residue dried for one hour in the oven at 90° C., cooled and weighed.

EXPERIMENTAL DATA AND DISCUSSION

The results of these experiments are shown graphically and in tables on the following pages.

The number and lengths of roots and length of plumules for the three stages are shown in Table 1. There was an average of five roots to each plant in each stage of growth. In each stage the average secondary root length was about 65 per cent of the primary root length. In stage I the plants had an average plumule length of 0½ inches; that of stage II was 9½ inches, and that of stage III was 8 inches. It will thus be noticed that the length of the plumules of the 10-day seedlings is greater than that of the 16-day plants, while the root length of the 10-day plants is less than that of the 8-day ones. This may have been due to the fact that the 10-day stage had been watered more frequently than either of the other two stages.

Table I.--Number and length of roots and plumules at three stages of growth of corn seedlings.

	I 6-day Feb. 11-19	II 10-day Feb. 14-24	III 14-day Feb.24 - Mar.10
Number of roots	4-6	3-8	3-8
Average	5	6	6
Primary root length in inches	7-9	5-15	8-12
Average	8	7	9½
Secondary root length in inches	4-6	3-9	4-12
Average	5	4 3/4	7
Plumule length in inches	3-9	7-14	7-11
Average	6½	9½	9

Only 36.65 per cent of the original dry matter of the endosperm remained after 14 days of germination. At that time the total dry weight of all parts was but 66.30 per cent of the original dry weight of the whole grain. Over a third of the original amount of dry matter had been used up in respiration. This is shown in Table II and graphically in Figure 1.

Table II.--Percentage of the original dry matter of endosperm and of plant parts after 8, 10, and 14 days in the germinator; the dry ungerminated seed and endosperm each represent 100 per cent.

	Dry seeds	8-day	10-day	14-day
Endosperm	100	73.24	54.38	36.05
Total parts	100	90.97	75.53	65.39

Table III.--Grams of dry matter per 100 plants or plant parts.

	Dry seeds	8-day	10-day	14-day
Endosperm	29.7644	22.0000	16.3504	10.9174
Root		2.7036	2.2760	4.5456
Plumule		5.0564	5.9758	5.7702
Mesocotyl		0.5389	0.5074	0.5440
Embryo	5.5402			
Total	35.3046	30.2989	25.0896	21.7772

Table III shows the increase or decrease in dry weight per 100 of the various plant parts. The endosperm loses weight steadily in each successive stage. The weight of the roots of 100 plants of stage II was less than that of 100 plants of stage I, and only about half that of stage III, while the weight of the plumules of 100 plants of stage II was slightly greater than that of stage III and

about 20 per cent greater than that of stage I. The weights of the cotyledons of 100 plants of each stage did not vary to any marked degree. Figure 1 also shows the grams of dry matter per 100 plants in each stage of growth.

The total nitrogen content of 100 plants was a little less at each successive stage. There was a two per cent loss after eight days in the germinator. For the 10-day stage there was a six per cent loss of the original amount, while after 14 days the loss amounted to 9.5 per cent, or 66 milligrams of an original total of 692 milligrams. This is contrary to results usually found by investigators for seeds rich in carbohydrates. A possible explanation might be found in the leaching out of the nitrogen by the water used in keeping the germinators moist. When the dolls were moistened, water was allowed to flow freely over and through them until they were well soaked. The excess water was allowed to drain off as waste. It would be interesting if an analysis had been made of the water before and after its use on the rag dolls. At the end of the 14-day period there was nearly five grams of starch left in the 100 residual seeds (Tables III and V), so it is quite unlikely that the protein was used in respiration to such an extent as to account for the loss. A study of Table IV indicates the greatest loss of nitrogen of the embryos from stage II seedlings, the ones that received

the greater number of waterings. The amount of nitrogen in the roots and plumules at this stage was not materially less in proportion to dry weight than would be expected, according to the amounts secured in stages I and II. Figure 2 shows a lower percentage of nitrogen in the roots in each successive stage. The same is true of the mesocotyl. The percentage of nitrogen in the plumule increased, although, as shown in Table IV, the largest amount of nitrogen per 100 plant plumules was found in the 10-day stage. Nearly 75 per cent of the nitrogen of the original seed has been translocated to the roots and plumules at the end of 14 days. The earlier seedlings had a greater per cent of nitrogen in the roots and tops than the later seedlings.

The total loss of nitrogen from the seedlings in eight days was 14.42 milligrams; in ten days it was 42.73 milligrams, and in 14 days it was 65.98 milligrams.

The percentage of nitrogen in the whole dry corn was 2.08--a protein content of approximately 13 per cent.

Table IV.—Changes in the nitrogen content of the dry matter of the seeds and seedlings at three stages of growth.

	Dry seeds	5-day	10-day	15-day
	Percentage			
Endosperms	1.98	1.32	1.46	1.52
Roots		3.08	3.40	3.16
Plumules		5.22	5.23	5.13
Hesocotyls		4.86	4.49	4.02
Embryos	2.97			
	Milligrams in 100 seeds and seedlings			
Endosperms	588.6	251.1	238.9	166.5
Roots		99.7	77.4	143.5
Plumules		265.8	312.3	297.3
Hesocotyls		25.1	22.8	21.9
Embryos	105.6			
Total	694.2	670.7	651.4	628.2

The percentage and amount of starch were computed on the basis of d-glucose. The reserve of starch in the endosperm was rapidly depleted during the 14 days, less than 19 per cent of the original amount remaining. The depletion was especially rapid between the eighth and tenth days. Less than eight per cent of the original amount of starch was found as such in the roots and plumules. There was a greater percentage of starch in the roots than in the plumules; about 24 per cent of the dry weight of the roots and 18-24 per cent of the dry matter of the plumules was starch. The embryos and mesocotyls were not tested for starch because of such a small quantity of material in these parts.

Table V.--Amount of starch (basis of d-glucose) in seeds or plant parts at three stages of seedling growth.

	Dry seed	8-day	10-day	14-day
	Percentage			
Endosperm	94.42	80.93	64.34	48.04
Roots		24.14	23.66	25.62
Plumules		20.68	18.42	18.14
	Milligrams per 100 seeds or plant parts			
Endosperm	28,391.0	17,815.6	8,059.9	3,564.7
Roots		652.6	524.6	1,164.8
Plumules		1,047.7	1,100.7	1,040.7
Total	28,391.1	19,515.9	10,515.4	7,456.0

Table VI shows the results of the sugar tests. The total sugars for 100 seedlings of stage I were more than double that of the dry seed. For each succeeding stage the amount was about the same as for stage I. This increase over the dry seed is accounted for by hydrolysis of the starch. The endosperm showed an increase in the percentage of sugars for each successive stage, but the actual amount in the residual endosperm was much smaller with each succeeding stage. The reserve food of the endosperm is rapidly depleted as new cells are formed in the plumule

and roots and energy is released in this activity. The mesocotyls and plumules showed the largest increase in percentage and actual amount of sugars (Fig. 4). The roots were the only parts that showed a decrease in percentage of sugars for the three stages, the percentage here ranging from 4.4 to 1.3%. The total amount of sugars per 100 plants decreased 73.7 milligrams between the eighth and tenth days, but increased again slightly by the fourteenth day.

No reducing sugar was found in the embryo and very little in the endosperm of the dry seed (Table VI). The embryo had considerable non-reducing sugar, 240.3 mg. per 100 embryos. Only 107.7 mg. were present in 100 endosperms. The 8-day endosperm had 100.0 mg., the 10-day had 113.9, and the 14-day had 171.4 mg. There was a large increase in the amount of reducing sugar in the endosperm during germination. From 41.7 mg. per 100, it increased to 770.8 mg. in the 8-day endosperm, to 798.6 in the 10-day endosperm, and then decreased to 400.8 mg. in the 14-day endosperm.

The roots showed a decreased amount of both sugars in the 10-day stage, as compared to the 8-day plants and a slightly increased amount in the 14-day stage. The plumules showed an increased amount in successive stages

for reducing sugars, being 58, 65, and 44 mg. per 100 for the 8, 10 and 14-day plants respectively.

In general the total non-reducing sugars of the entire plant showed a rapid falling off to the tenth day and then a moderate increase. The total reducing sugars of the entire plant increased markedly for the first stage, going from 41.7 mg. per 100 seeds to 311.6 mg. per 100 plants eight days in the germinators. For the 10-day stage there were 905.4 mg. and 912.5 for the 14-day stage.

Only small amounts of non-reducing sugars were found in the various parts of the seedlings at the different stages. Thus only 0.55 per cent of the dry endosperm was non-reducing sugar. About 0.83 per cent of the 8-day endosperm, 0.71 per cent of the 10-day endosperm, and 1.07 per cent of the 14-day endosperm were non-reducing sugars. Non-reducing sugars made up 2.03 per cent of the 8-day roots, but only 0.38 and 0.40 per cent, respectively, of the 10-day and 14-day roots. The plumules contained 0.42 per cent to 1.17 per cent non-reducing sugars.

Table VI.--Amount of reducing, non-reducing, and total sugars (basis of D-glucose) for dry seed and three stages of seedling growth.

	Dry seed			5-day			10-day			15-day		
	Reducing	Non-reducing	Total	Reducing	Non-reducing	Total	Reducing	Non-reducing	Total	Reducing	Non-reducing	Total
	mg	mg		mg	mg		mg	mg		mg	mg	
	Percentage											
Endosperm	0.55	0.14	0.07	0.83	3.04	4.37	0.71	4.50	5.00	1.57	4.25	5.80
Roots				5.63	1.77	4.40	0.58	1.21	1.74	0.46	10.05	1.31
Plumules				1.17	1.08	2.70	0.42	2.70	3.17	0.70	10.01	7.00
Mesocotyls				1.01	0.00	2.07	2.06	0.00	2.06	0.27	2.70	10.27
Embryos	9.81	-	9.81									
	Milligrams per 100 seeds or plant parts											
Endosperms	167.7	41.7	109.4	255.6	778.2	901.4	116.9	738.0	914.5	171.4	460.6	632.0
Roots				71.1	47.9	119.0	15.1	47.0	62.0	20.9	30.0	69.0
Plumules				29.2	11.9	141.1	25.1	106.3	150.4	44.0	303.0	457.0
Mesocotyls				3.1	3.0	11.1	10.4	3.0	13.4	33.7	30.1	63.8
Embryos	348.5	-	348.5									
Total	300.0	41.7	347.7	381.0	911.0	[illegible]	165.3	993.6	1158.3	870.0	914.6	1184.5

Table VII and Figure 6 show rather uniform values for the percentage of ether extract in the residual seeds, the roots and the plumules of the three stages. The percentage of ether extract in the mesocotyl was higher, being 5.36, 5.85, and 6.97 per cent, respectively, for the three stages. The percentage of ether extract of the whole seed was 6.15, nearly 70 per cent of it in the embryo. The embryo yielded 30.69 per cent of its dry weight as ether extract. Only one per cent of the dry weight of the endosperm was ether extract.

One hundred endosperms lost about 100 milligrams of ether extract for each successive stage, accounted for by transformation, translocation, and respiration. One hundred plumules had 117.6, 193.6, and 217.0 milligrams of ether extract for respective stages of development.

There were 1387.1 milligrams of ether extract in 100 dry kernels of corn. At the 6-day stage there were 929.5 milligrams; at the 10-day stage there were 831.9 mg.; and at the 14-day stage 760.8 mg. ether extract, a total loss in 14 days of 45 per cent.

Table VII.--Amount of ether extract in seeds or plant parts at three stages of growth.

	Dry seed	5-day	10-day	15-day
	Percentage			
Endosperms	1.00	2.37	3.25	3.69
Roots		2.20	2.53	1.58
Plumules		2.38	5.24	3.76
Mesocotyls		3.86	9.85	6.97
Embryos	30.69			
	Milligrams per 100 seeds or plant parts			
Endosperms	207.5	681.4	530.7	424.7
Roots		88.3	87.6	72.6
Plumules		117.8	188.6	217.0
Mesocotyls		20.9	50.0	36.5
Embryos	1099.6			
Total	1307.1	909.6	851.9	750.8

SUMMARY

The purpose of the problem was to find out by chemical analyses some of the organic changes that take place in the corn kernel, Zea mays, during germination, and to find the amounts of the organic materials in the seedlings at different stages, compared with the original amounts in the kernel.

Pride of Saline corn, shelled by hand from thirty selected ears, was carefully graded and sorted before use. The seed was treated with a commercial fungicide just prior to its use in the germinators.

Rag doll germinators were used because of the ease of growing the seedlings without sand, and because the dolls could be placed in vessels from which light could be completely excluded.

Seedlings were grown to three stages: 8-day, 10-day, and 14-day stages. The plumules, roots, mesocotyls, and residual seeds of healthy plants were separated, dried, and analyzed for nitrogen, carbohydrates, and fats.

Only 35 per cent of the original dry matter in the endosperms remained in the residual seeds after 14 days of germination. Nearly 35 per cent of the original whole grain was used or lost through respiration in 14 days.

The nitrogen loss amounted to 2, 6, and 9.5 per cent for stages I, II, and III, respectively. This loss was probably due to leaching by the water used in moistening the rag dolls. Nearly 75 per cent of the nitrogen of the original seed had been translocated to the roots and plumules at the end of 14 days. Over half of the nitrogen of the endosperm was translocated to the roots and plumules during the first eight days, while during the same time

55 per cent of the nitrogen of the whole dry kernel was translocated.

The 14-day endosperm contained but 10 per cent of its original starch. The starch of the 14-day roots and plumules was about 6 per cent of the starch of the original seed. Less than 27 per cent of the original starch was found in the plant at the end of 14 days. About 24 per cent of the dry weight of the roots and 18-24 per cent of the dry matter of the plumules was starch.

The total sugars of the seedling plants were more than double that of the original seed. The increase is accounted for by hydrolysis of starch and fats. After the first stage, the roots showed a decrease in their sugar content. No reducing sugar was found in the embryo and very little in the endosperm of the dry seed. One hundred embryos contained 348.3 mg. of non-reducing sugars; 100 endosperms contained 157.7 mg. of non-reducing sugars. The starch of the endosperm is hydrolyzed faster than the reducing sugars are used up and as a result non-reducing sugar as a temporary storage product is increased somewhat in the residual seeds. Reducing sugars are increased from 41.7 mg. per 100 original endosperms to 773.0 mg. per 100 8-day residual seeds, and 793.6 mg. per 100 10-day residual seeds. The reducing sugars have decreased to 600.0 mg. per 100 residual

seeds at the 14-day stage. Total non-reducing sugars for the whole plant declined in amounts during germination, while the reducing sugars increased greatly to an early maximum, which was maintained until the 14-day period. Only small amounts of non-reducing sugars were found in the various parts of the seedlings at the different stages.

Ether extract was quite uniformly distributed in the seedlings of the three stages. Fats were translocated more slowly than carbohydrates and proteins. One hundred endosperms lost about 100 mg. of ether extract for each successive stage. Increase in sugars was no doubt augmented by the transformation of fat into these compounds. The percentage of ether extract of the dry seed was 4.15; 30.09 per cent of the dry weight of the embryo and one per cent of the dry weight of the endosperm was ether extract. Forty-five per cent of the ether extract of the corn kernel was used during 14 days in the germinators.

LITERATURE CITED

1. Chittenden, R. H., and Osborne, T. B.
1891-1893. The proteids of the wheat or maize kernel.
Amer. Chem. Jour. 13:453-468, 529-552; 14:30-44.

2. Choate, Helen A.
1921. Chemical changes in wheat during germination.
Bot. Gaz. 71:409-420.

3. Davidson, Jendel.
1926. Changes in nitrogen, phosphorus, and potassium content of wheat seedlings during germination and early stages. Bot. Gaz. 81:87-95.

4. Hopkins, C. G.
1898. Chemistry of the corn kernel. Ill. Agric. Exp. Sta. Bul. 53.

5. Jodidi, S. L.
1926. Nitrogen metabolism in etiolated corn seedlings. Jour. Agric. Res. 31:1149-1160.

6. _____
1927. Isolation and identification of some organic nitrogenous compounds occurring in etiolated corn seedlings. Jour. Agric. Res. 34:643-651.

7. Le Clerc, J. A., and Breazeale, J. F.
1911. Translocation of plant food and disintegration of organic plant material in wheat seedlings. Bur. Chem., U.S.D.A. Bul. 138.

8. Latshaw, W. L., and Miller, E. C.
1924. Elemental composition of the corn plant. Jour. Agr. Res.27:845-859.

9. Miller, E. C.
1910. Physiological study of the germination of Helianthus annuus. Annals of Bot. 24:733-788.

10. _____
1912. A physiological study of the germination of Helianthus annuus. The oily reserve. Annals of Bot. 26:953-991.

11. Newcomb, Margaret Alice.
1927. A study of the nitrogen changes in the germination of oily seeds. Mass. St. Agric. Col. Master's Thesis, 17:1-37.

12. Osborne, T.B.
1897. The amount and properties of the proteids of the maize kernel. Jour. Amer. Chem. Soc. 19:525-532.

13. _____
1908. Hydrolysis of the proteins of maize, 2nd paper. Amer. Jour. Physiol. 20:477-493.

14. Pettibone, S. J. V., and Dunnell, Cornelia.
1918. Translocation of seed protein reserves in the growing corn seedling. Jour. Bio. Chem. 33:519-526.

15. Shiaw, J. H.
1926. Translocation of fats as such in germinating seeds. Bot. Gaz. 82:154-189.

16. Sachs, J.
1882. Lectures on the physiology of Plants; Lecture XI. Oxford Clarendon Press, London.

17. Stark, C. E.
1927. Protein metabolism of the soy bean. Amer. Jour. Bot. 14:532-547.

18. Thompson, P. G.
1915. Total amino nitrogen in the seedlings of the Alaska pea. Jour. Amer. Chem. Soc. 37:230-238.

19. Toole, E. H.
1924. The transformation and course of development of germinating maize. Amer. Jour. Bot. 11:325-343.

20. Vozzo, A. E.
1925. Translocation of food materials of wheat seedlings. Jour. Agr. Res. 31:727-737.

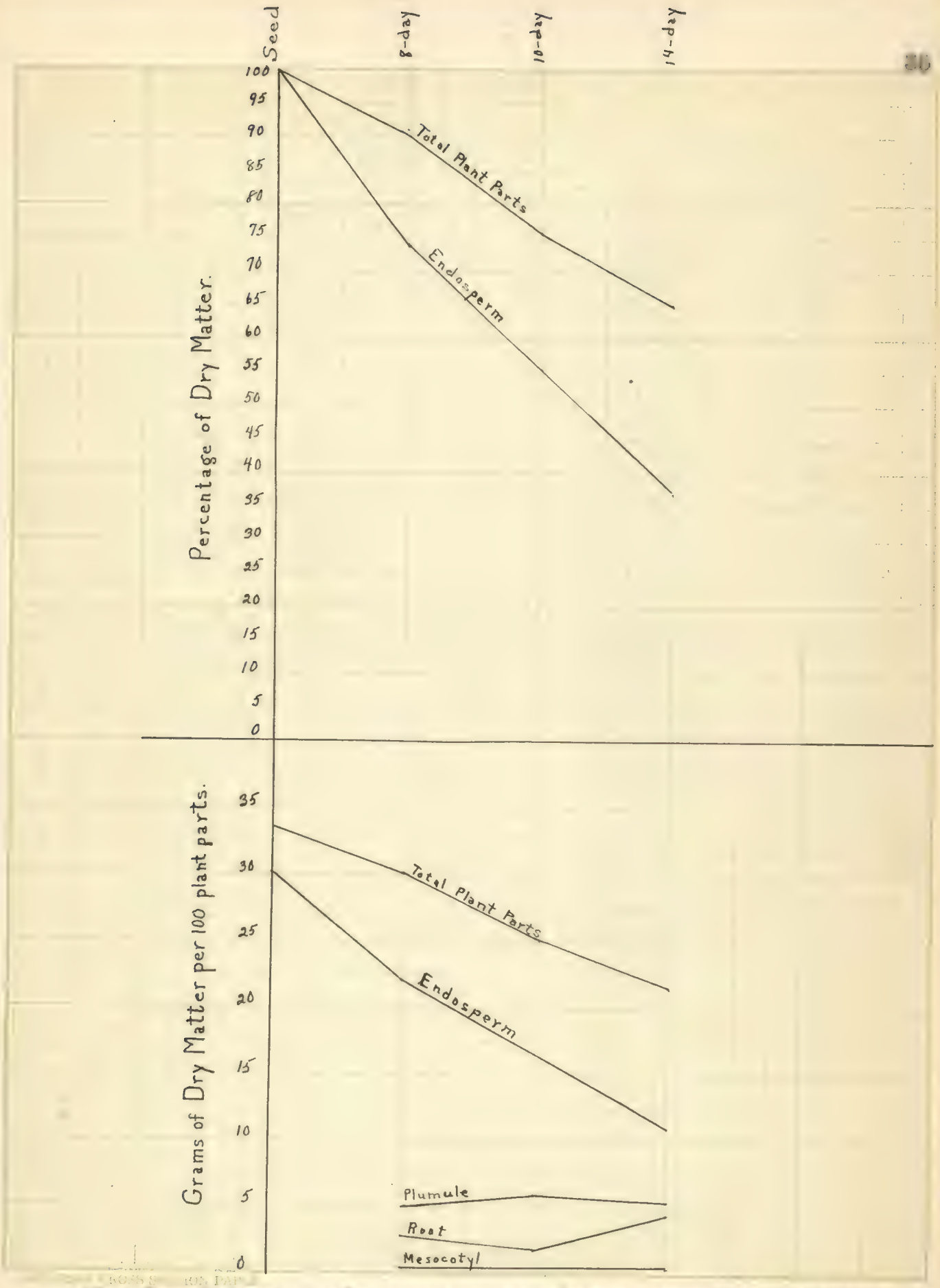

Fig. 1.--Graphs showing percentage of dry matter of endosperm and total plant parts, and grams of dry matter per 100 plant parts.

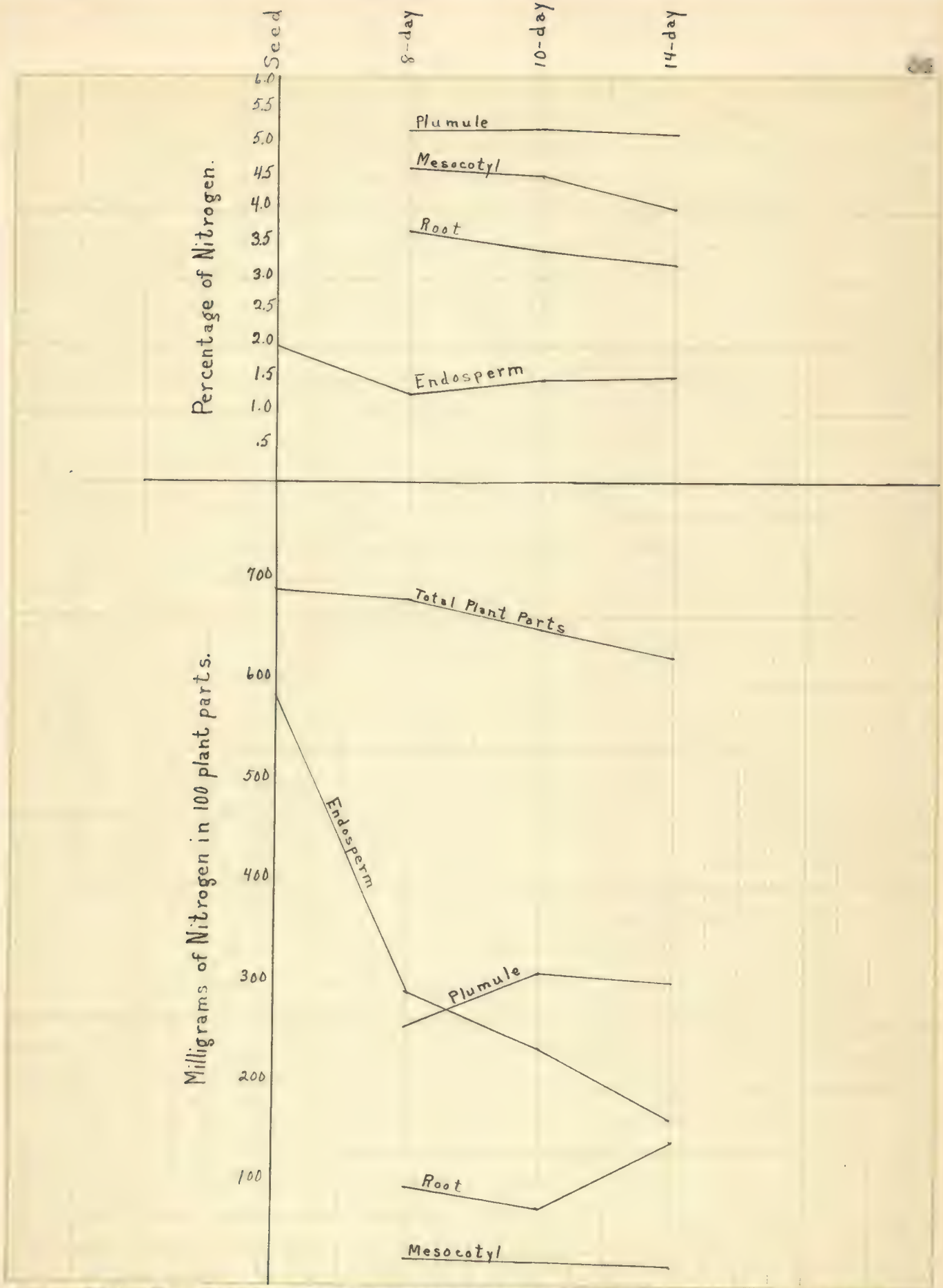

Fig. 2.--Graphs showing percentage of nitrogen in plant parts and the amount of nitrogen per 100 plants or plant parts.

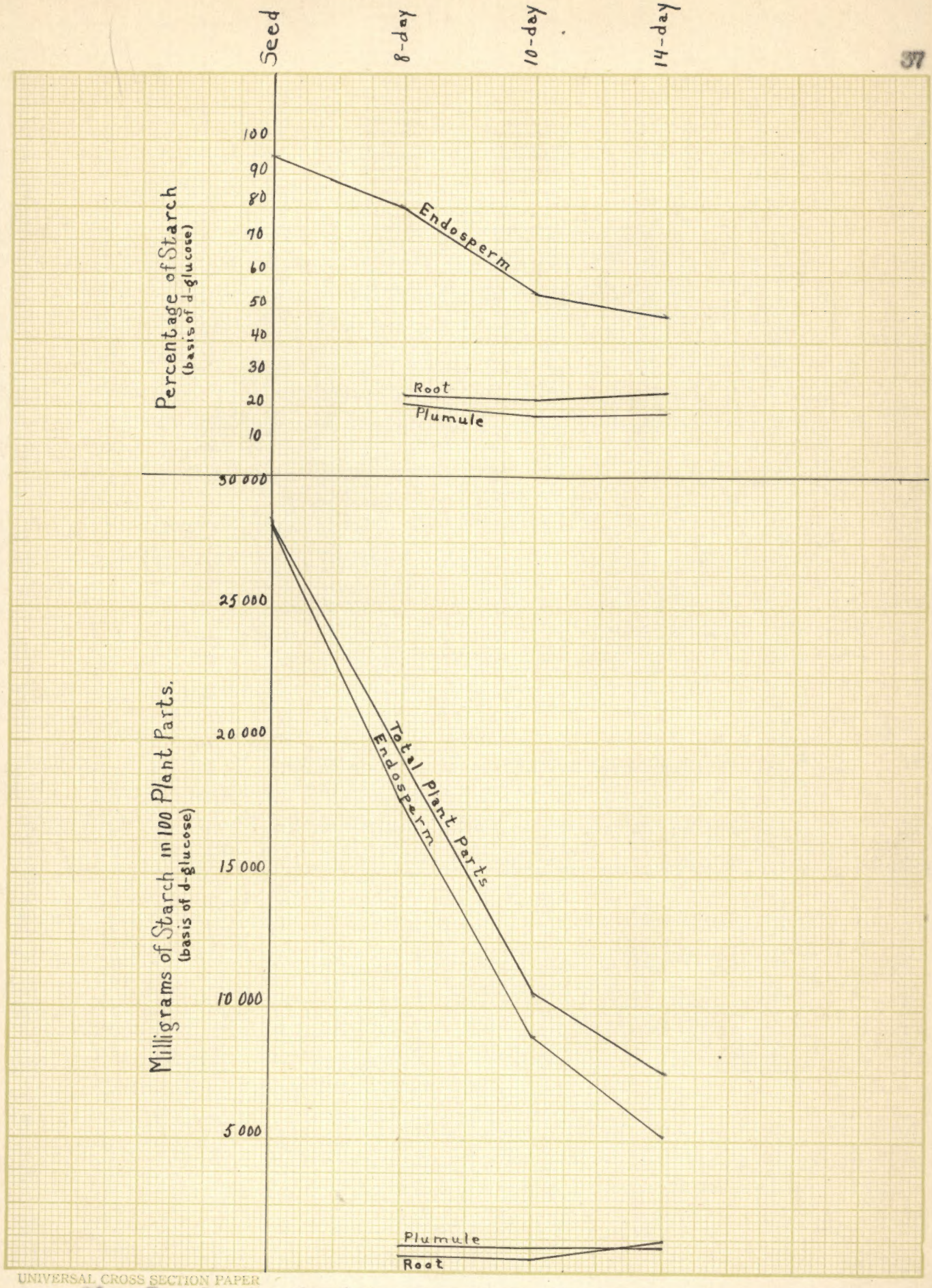

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Fig. 3.--Graphs showing percentage of starch and amount of starch per 100 plants or plant parts, all on the basis of d-glucose.

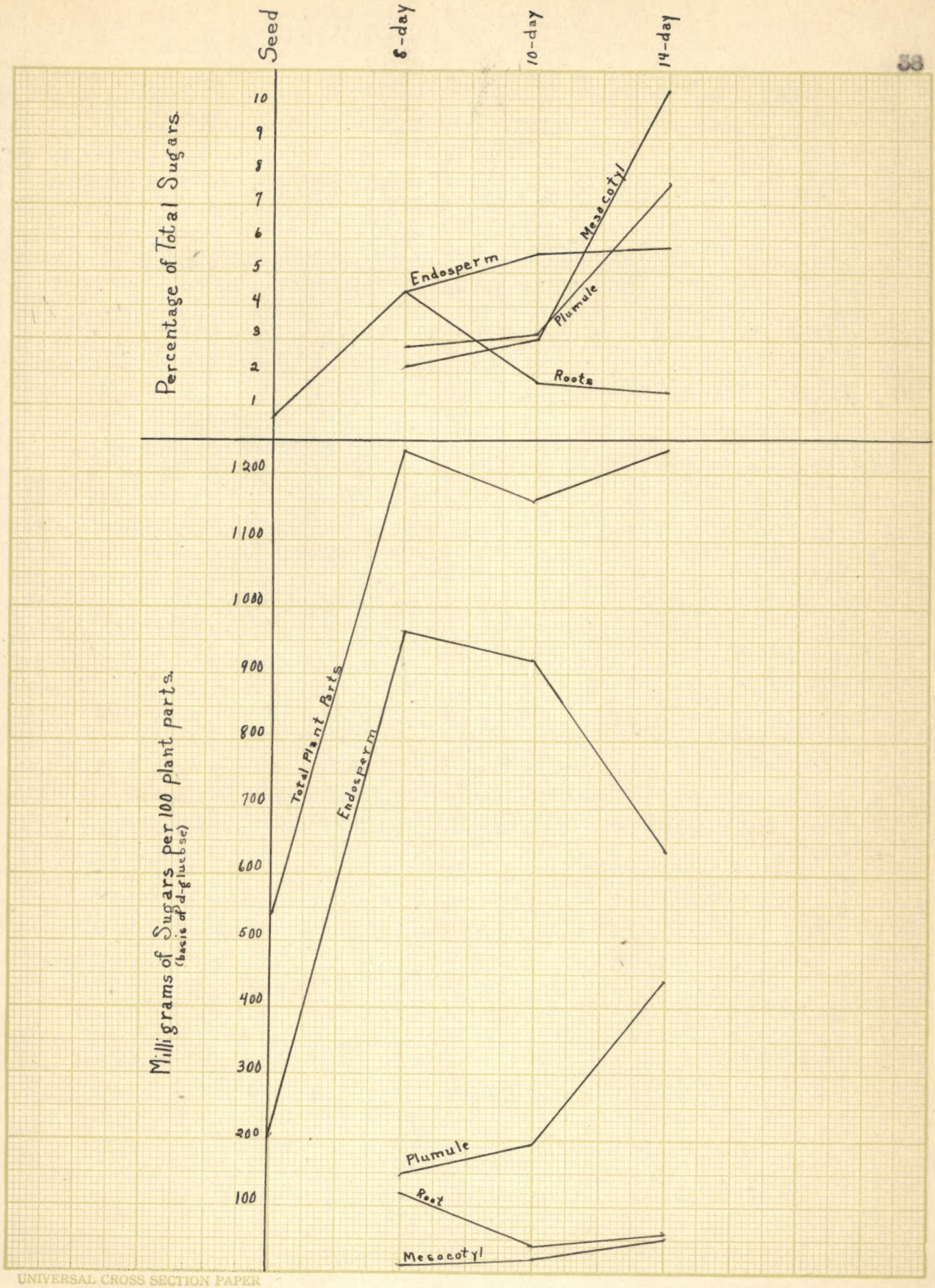

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Fig. 4.--Graphs showing percentage and amounts of total sugars per 100 plants or plant parts, basis of d-glucose.

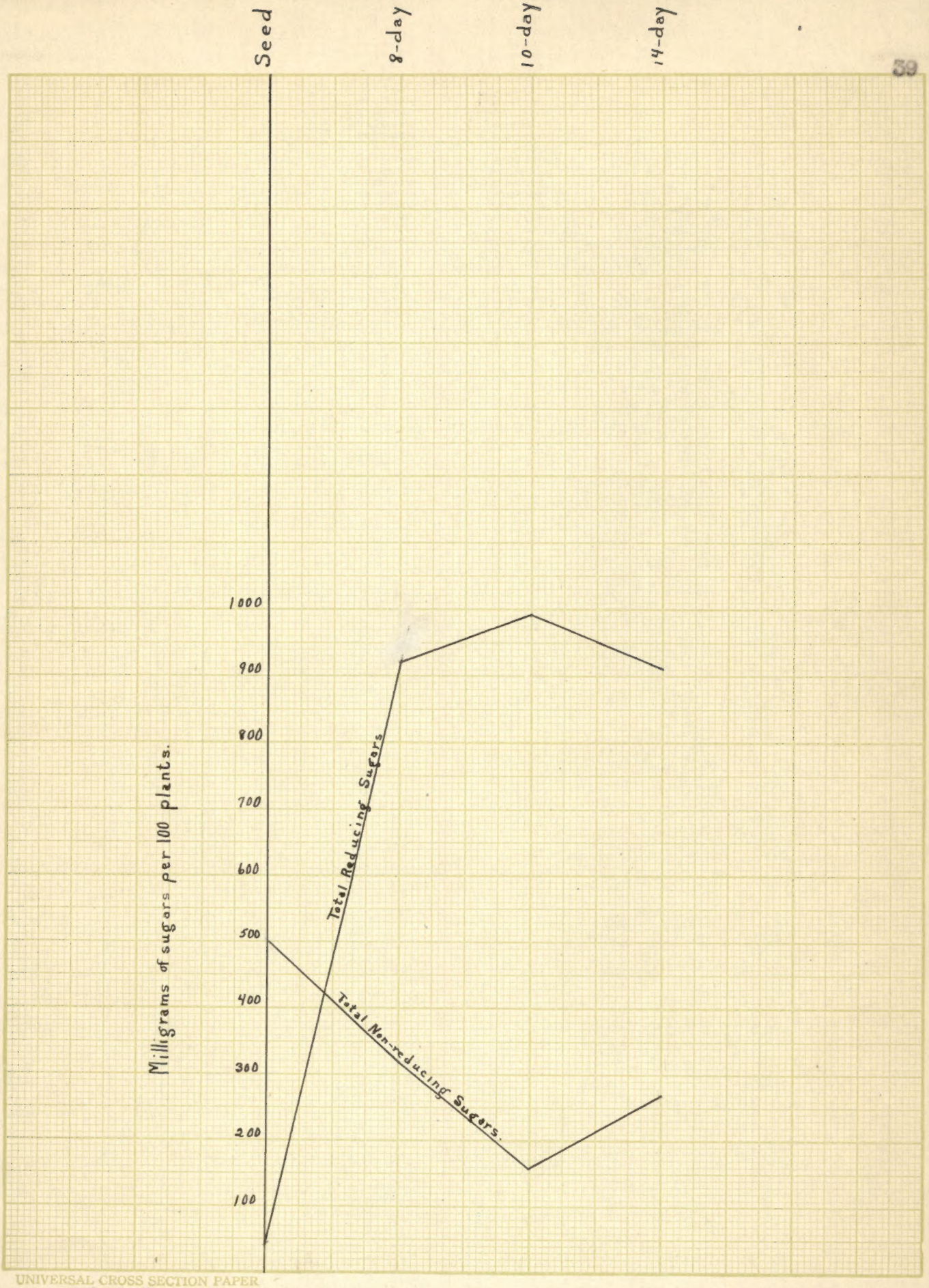

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Fig. 5.--Graph showing the total reducing and non-reducing sugars per 100 plants for three stages of growth, and in seed,--basis of d-glucose.

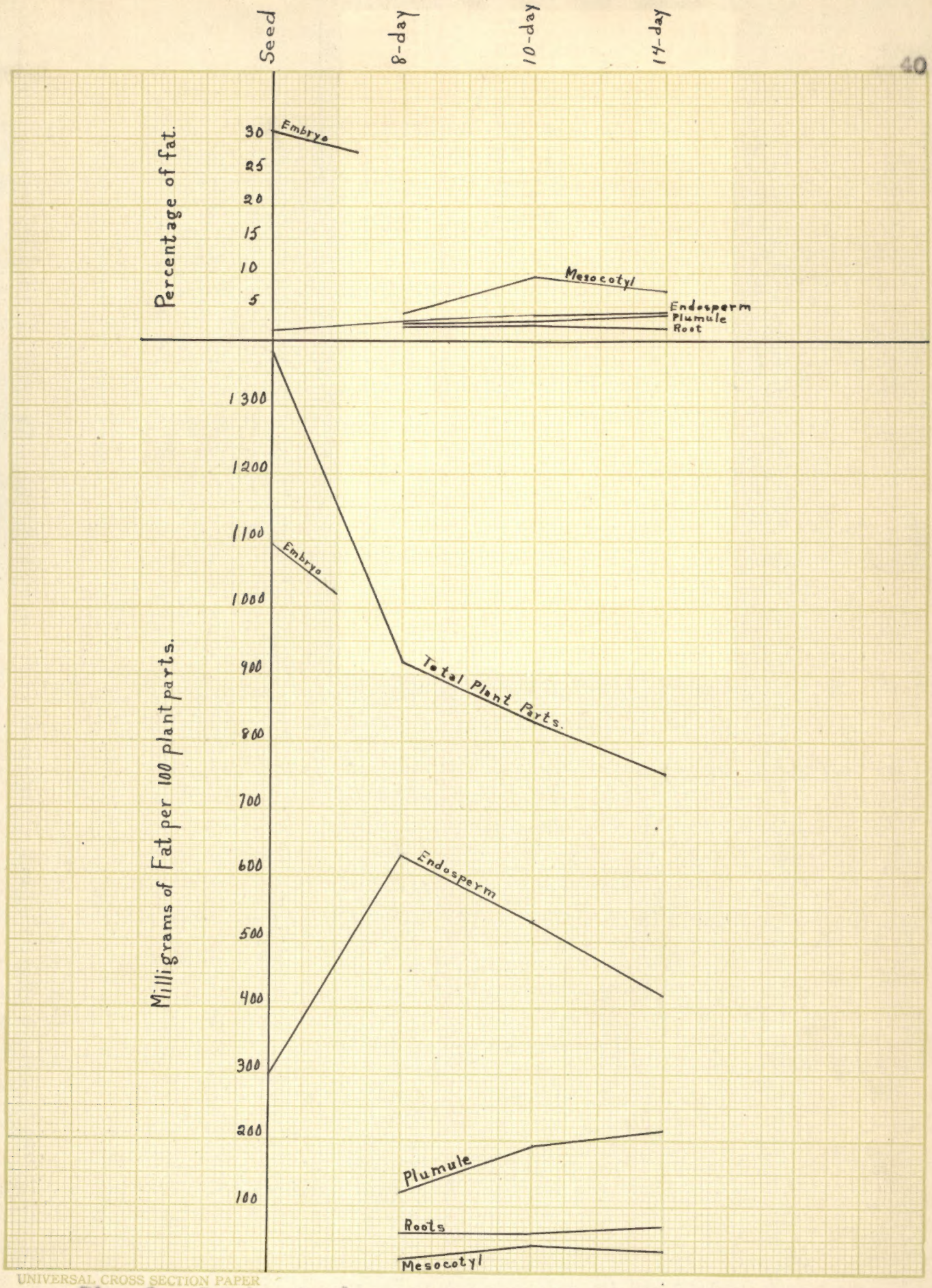

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Fig. 6.--Graphs showing percentage and amounts of ether extract per 100 plants or plant parts.